U. S. National Institute of Health

BULLETIN No. 7 OF THE HYGIENIC LABORATORY,
Treasury Department, U. S. Marine-Hospital Service,
WALTER WYMAN, Surgeon-General.
MAY, 1902.

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S. B. GRUBBS AND EDWARD FRANCIS.

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RING TEST FOR INDOL.

(BY S. B. GRUBBS, PASSED ASSISTANT SURGEON, AND EDWARD FRANCIS, ASSISTANT SURGEON, UNITED STATES MARINE-HOSPITAL SERVICE.)

The method of applying the indol test, as ordinarily used in laboratories and described in our text-books, is certainly crude, and consequently the statement that an organism does or does not produce indol leaves us in most cases very uncertain as to just what is meant.

As we know, the line between the indol producers and non-producers is not distinct, and several organisms "not producing indol" do give the reaction if grown long enough in suitable media. Thus, for example, we can get a faint but distinct reaction from cultures of Bacillus pestis and Bacillus typhosus, although these organisms under ordinary conditions are not considered indol producers.

So we must draw the line arbitrarily, and in this laboratory we take cultures grown twenty-four hours at 37° C. in media containing 1 per cent of peptone and use the ring or contact test, allowing it to remain in contact one hour.

We have applied the ring test in determining the presence of indol on account of its simplicity, its clearness, and its delicacy. When the acid and solution of nitrites were mixed, we found the color reaction was often unsatisfactory, the natural yellow tint masking the pink, or the color appearing on first contact with the nitrite solution would disappear when the tube was shaken.

The following technique will always give a clear-cut reaction if indol is present, or if not, there is no difficulty in deciding in the negative:

To 7 c.c. of the twenty-four-hour culture add 8 to 10 drops of concentrated sulphuric acid and shake; then let 3 or 4 c.c. of a 1–1000 sodium-nitrite solution run carefully down the side of the tube so as to form a sharp line of contact between the heavier culture and acid and the lighter nitrite solution, when the pink color will contrast sharply with the portions above and below.

The adoption of this technique, with the time limits mentioned, will make the indol reaction very clear in its results and will increase its value as a means of recognizing species.

COLLODIUM SACS.

(BY S. B. GRUBBS, PASSED ASSISTANT SURGEON, AND EDWARD FRANCIS, ASSISTANT SURGEON, UNITED STATES MARINE-HOSPITAL SERVICE.)

Since the publication of the work of Metchnikoff, Roux, and Salimbeni[1] on the toxins of cholera, the growing of cultures in collodium sacs placed in the peritoneal cavities of animals has become a common procedure not only at the Pasteur Institute, but in all bacteriological laboratories when it is desired that the culture in its growth shall be raised in virulence through the action of the nutrient body fluids and yet be protected from the influence of the body cells and phagocytes.

As usually made, the sac is molded over the end of a glass rod by dipping it several times into the collodium solution, after which it is peeled off (Pasteur Institute).

Novy[2] slips inside the sac a glass tube which is perforated and constricted, and he fixes the free border of the sac to the glass tube at a point just below the constriction by means of heat, pressure, a silk ligature, and additional collodium.

McCrae,[3] following Prudden, employs a glass tube, to the heated end of which he attaches a gelatin capsule which acts as a temporary frame over which the collodium is molded. The capsule is then removed by dissolving it in hot water.

In the techniques just referred to there are several delicate manipulations which greatly hazard the chances of getting a perfect sac:

1. There is the danger of tearing and destroying the sac in peeling it off the rod.
2. There is considerable difficulty in attaching the sac securely to the glass frame; and,
3. If no frame is used, the sac has no support, may become shriveled, and is liable to tear on removal from the animal's abdominal cavity if bound around by adhesions.

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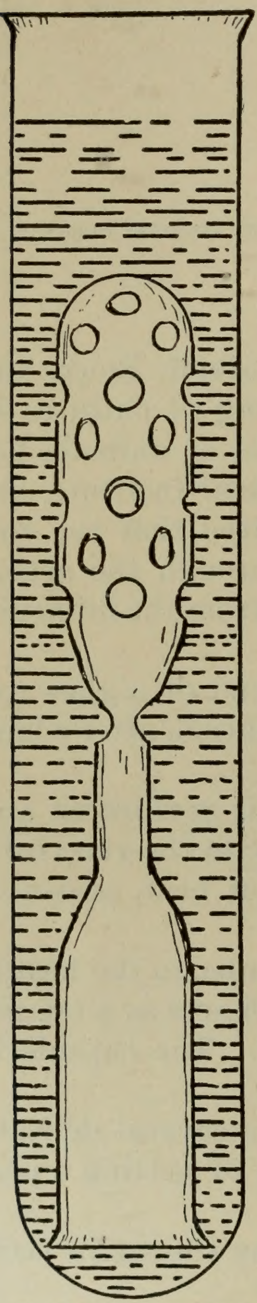
[1] Toxine et antitoxine cholerique Ann. de l'Institut Pasteur 1896, X.

[2] Laboratory work in bacteriology.

[3] Journal of Experimental Medicine, vol. 5.

In endeavoring to overcome these difficulties we have hit upon the following simple technique and have used it repeatedly in the Hygienic Laboratory for several months:



The glass frame inclosed in a test tube of hot water to remove the gelatin from the perforations.

The sac is molded in the very beginning over the perforated glass frame, from which it is not later removed.

The steps are as follows:

A glass frame is first made according to the method of Novy. Eight to twelve openings, each having a diameter of about $\frac{1}{8}$ inch, are blown as closely as possible in the lower part of a small test tube, the size and number of the holes depending on the amount of osmosing surface desired.

The tube is then partially constricted in the flame at a point just above the openings. The constriction is of such size as to admit a fine pipette for inoculation. Some pure gelatin, to which is added a little water, is liquefied by heat, and with a glass rod a drop of the hot gelatin is applied to each individual opening in the glass frame, which, when it solidifies, forms a thin film which temporarily closes each perforation.

The tube is now dipped two or three times into the collodium solution to a point well above the openings but below the constriction. In order to remove the gelatin film from the holes, the tube is filled with water and it is inverted in a larger tube or beaker of water the temperature of which is raised until the gelatin melts and runs down the inside of the tube through the constricted part and out into the water (see cut).

We then have the collodium firmly adherent to the glass frame, covering its openings, well supported and ready to stand, without tearing or leaking, the manipulations of sterilization, filling, inoculation, sealing, implantation and removal from the abdominal cavity.

The ease with which this technique can be carried out will, we think, appeal to those who have had experience with other methods.[1]

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[1] The glass frame may be further improved by directing the gas blast flame against the tube on opposite sides just below the constriction until the glass becomes softened and indented. These two flattened surfaces afford a good place for grasping the frame with a pair of forceps during the sealing off and implantation. This overcomes the slipping and turning which occur on attempting to hold firmly a perfectly round tube.

MICROPHOTOGRAPHY WITH SIMPLE APPARATUS.

(BY H. B. PARKER, ASSISTANT SURGEON, UNITED STATES MARINE-HOSPITAL SERVICE.)

The art or mechanics of photography has in the last few years become very useful in pathological, histological, and bacteriological work. Almost every laboratory is supplied with an approved apparatus, while the independent workers convert their photographic apparatus, either kodak or camera, into some form of apparatus to meet their particular desires or needs. As a reward for their efforts they meet with varying degrees of success, so much so that in modern medical literature it is impossible in many instances to determine from the results whether the writer had in his possession an approved model or a simple makeshift of his own.

With low diameters almost any combination of microscope, camera, and illuminant will produce a fair picture in the hands of an amateur familiar with outdoor photographic processes. To obtain success with the higher diameters special apparatus is required, and these, I regret to say, are so expensive and difficult of manipulation that their use is prohibited in any but the more luxuriously equipped laboratories. The average size of the picture desired in bacteriological work is 2 inches; for pathological work somewhat larger; the average size of the plate in the modern microphotographic outfit is 6½ by 8½ inches to 8 by 10 inches with a bellows extension that will average 5 feet. This long extension was for a time an advantage, permitting the use of low-power objectives, amplification being secured by distance, but with the advent and perfection of apochromatic objectives, compensation and projection oculars, and instruments of greater precision in sectioning pathological and histological processes, the use of the long bellows is practically limited to achromatic and specially devised photographic objectives. A disadvantage probably more prominent than the preceding is the necessity of an arc light with its elaborate system of condensers and absorbing cells as the illuminant, a system too complex for the small and moderate size laboratory.

To overcome some of the disadvantages mentioned we have constructed an apparatus on the usual principle of the horizontal instrument but have reduced the size of the bellows extension to 32 inches and the size of the plate to 4 by 5 inches. We used an ordinary lantern-slide camera, which gave the results shown in the illustrations.

DESCRIPTION OF THE APPARATUS.

Success in microphotographic work depends largely upon the illuminant, of which there are six for consideration: Sunlight, the electric arc light, the oxyhydrogen light, illuminating gas, coal oil, and acetylene. Sunlight gives excellent photographs but is excluded on account of the expensive apparatus and special requirements for good work. The electric arc gives excellent results and is necessary when magnifications above 1,500 diameters are desired. The oxyhydrogen light, illuminating gas, and coal oil are too feeble for any but low diameters. Acetylene, because of its brilliancy, high actinic properties, low heat reflection, portability, low cost, and adaptability to other laboratory work, is the illuminant par excellence for diameters up to and including 1,500, for low-power microscopic projection, and lantern-slide projection.

The illuminating apparatus in use at this laboratory consists of a generator large enough to furnish gas for a quadruple burner, two condensers, of 4-inch focus, and a light filter and an absorbing cell holding a layer of water ½ inch thick. The latter is sufficiently large to remove all heat rays as well as serving to hold water tinged with a color complementary to the stain employed on the specimen to be photographed.

The bellows as described above is one of the numerous models for lantern-slide work, either 3¼ by 4¼ or 4 by 5 inches.

The whole apparatus is mounted upon a firm base.

FOCUSING.

One of the most difficult features in connection with microphotographic work is focusing the object on the ground-glass screen. We have discarded the various focusing rods used for this purpose and now depend upon a braided twine, the center going around the fine adjustment and thence to the back of the camera, where it is kept taut by small weights. This has the advantage that when the focus is accurately found the ends can be released, removing the strain from the fine adjustment and consequently all interference with the focus. Rods clamped to the fine adjustment are apt to change the focus when released and are acted upon by vibrations of the room.

A magnifying glass of low magnification can be used as an aid in focusing with high diameters, but as a rule the focus of the entire field can be much more accurately gauged with the eye alone than with the aid of lenses.

EXPOSURE.

The length of exposure will depend upon the intensity of the image upon the screen and upon the rapidity of the plate. A rapid exposure with an excess of light does not give the details which are obtained with less light and a longer exposure. In a general way then it may be said that the intensity of light should be subdued by reducing the diaphragm in the Abbe condenser until the exposure will approximately require about five seconds.

Care should be taken to see that no vibrations are produced during the exposure. This is best accomplished by selecting a time when the streets are free from passing vehicles, and all in the room should remain quiet. An ordinary pneumatic photographic shutter in the front board serves to reduce vibrations to a minimum and is far superior to the regular shutter supplied with these cameras.

CHOICE OF PLATES

Only plates of double coating should be selected for this work, preferably one of the medium or slow grades.

They are distinguished from the ordinary plates by their isochromatic or orthochromatic qualities, giving correct color values with double-stained preparations—a property that can be increased by using a color screen complementary to the object to be photographed.

As a rule with these plates, no color screen is required with fuchsin, safranin, hæmatoxylin, or carmine.

Without reference to the complementary color, a color screen made with chromic acid or sulphate of copper, or both, combined in weak solution will answer for any color or combination of colors in general laboratory use.

DEVELOPMENT.

A good developer has the property of producing both detail and density. A single developer does not possess these qualities perfectly, so a combination is used.

Metol and hydrochinone about represent the extremes, but when combined in proper proportions give the best results in microphotographic work, as well as in general photography. The formula most used is the following:

℞

Metol	2.00
Hydrochinone	2.00
Sodium sulphite	20.00
Sodium carbonate	10.00
Potassii bromidi	0.25
Distilled water	500.00

M.

The metol and hydrochinone should be dissolved in the water before adding the sulphite. This preparation keeps indefinitely, and may be used until exhausted. Whatever the exposure, the development should be carried out until the plate is completely developed; errors in over exposure or under exposure may then be corrected by reduction or intensification, either process with full development producing a better negative than one poorly developed.

Formulæ containing a mercury salt, for intensification, or containing potassium ferricyanide, for reducing, usually accompany all makes of plates.

COST OF TEMPORARY AND PERMANENT APPARATUS.

The advantage of this apparatus is the low primary cost. A lantern-slide camera at a cost of $10 makes an excellent bellows when firmly mounted on a frame. The generator, burner, two condensers and light filter would cost about $25, making a total cost of about $35.

A complete outfit with the necessary mechanical arrangements for centering the light, varying the focus of the condensers, and time shutter should be supplied for about $60.

Where a laboratory is supplied with electricity, that illuminant may be used by substituting a hand-feed electric arc and a large absorbing cell at an increase in cost of $5 to $10.

With this apparatus and the well-known apochromatic objectives and projective oculars of Zeiss, it is possible to take a photograph of 1,000 diameters in two seconds, or about as rapidly as an exposure can be accurately made. For the low diameter the light is stopped down until the desired illumination is obtained. Our experience is that an exposure of five seconds for all magnifications gives greater detail than a more rapid exposure. In this time vibrations do not seriously interfere with sharpness of outline. A printing-out silver paper gives the best tone to a photograph when half-tone reproduction is desired. The finished print should be a shade darker, as density is always lost in the process of reproduction.

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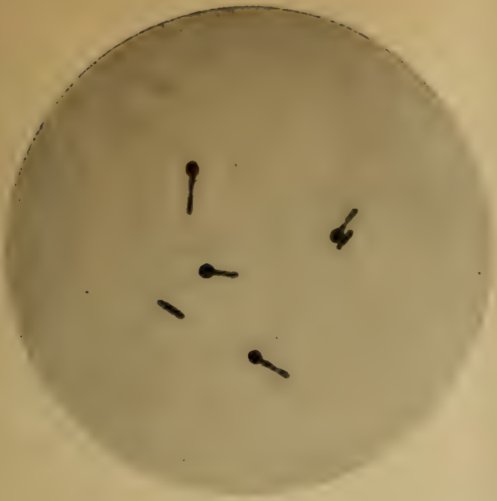
EXPLANATION OF PLATE No. 1.

1. Tetanus bacilli, with spores.
2. Typhoid bacillus, with flagella.
3. Plasmodium malariæ, in human blood, crescentic forms.
4. Staphylococci in pus.
5. Normal kidney, mouse.
6. Vaccine bodies in cornea of rabbit.

PLATE No. 1.



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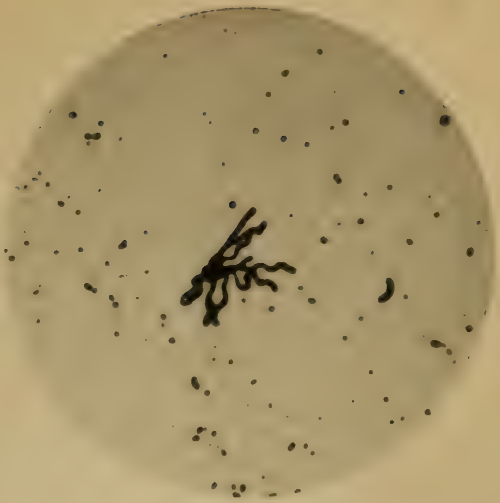

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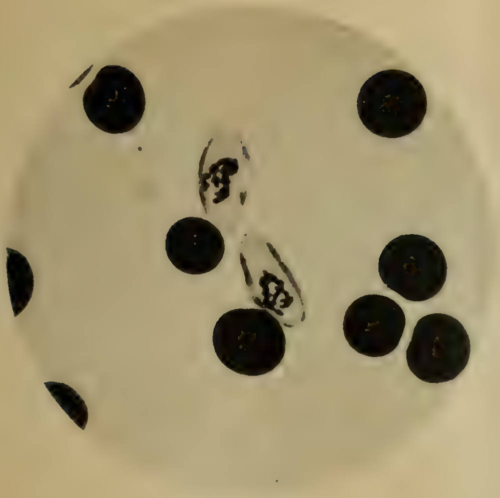

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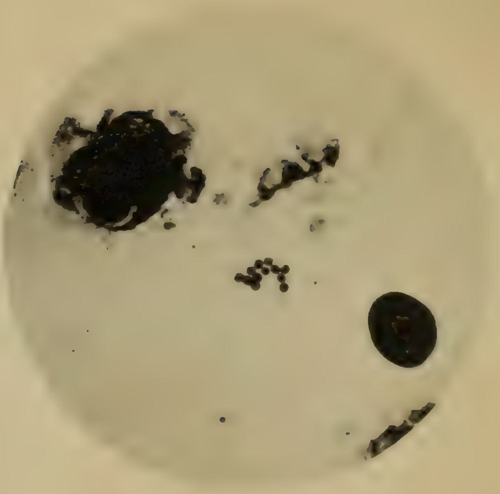

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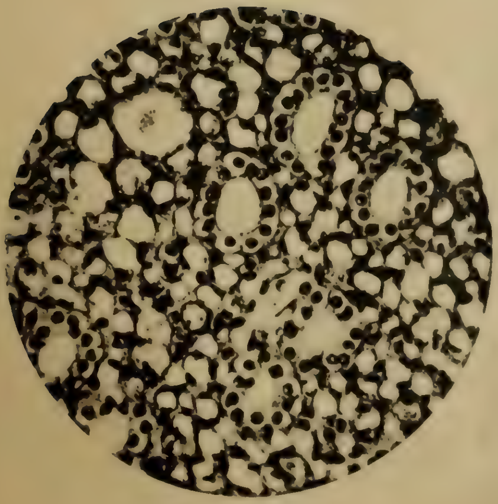

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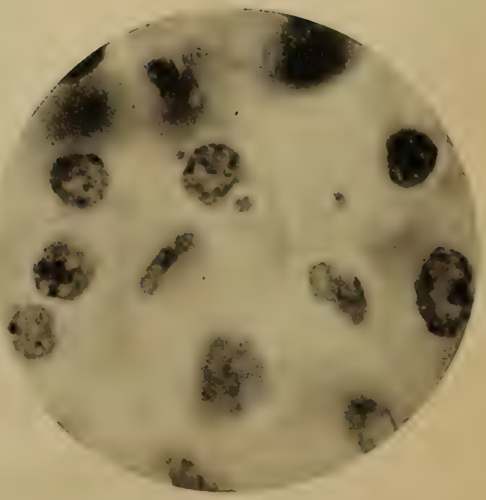

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HELIOTYPE CO., BOSTON

PLATE No. 2.